Detection of Cox2 in Formalin-Fixed, Paraffin-Embedded Rat and Mouse Tissue

Reagents:
1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information:

Blocking Serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #005-000-001

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #SP-2001

Primary antibody: Rabbit anti-mouse Cox2
Caymen Chemical
Ann Arbor, MI 48108
www.caymanchem.com
1-800-364-9897
Catalog #160106

Negative control serum: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Concentration: 60 mg/ml
Catalog #011-000-001

Secondary antibody: Biotinylated goat anti-rabbit IgG
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #BA-1000

Label antibody: Vector EliteVectastain® ABC
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6100

Staining Procedure:

-Positive Control Tissue: Highest level of expression located at the distal vas deferens where it inserts into the bladder. Weak staining at the proximal end of the vas defereas.

-Stain localization: Peri-Nuclear/Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Perform Heat Induced Epitope Retrieval using Microwave Oven.
Place a full rack of slides in Tissue Tek™ container containing 200 mls 1X citrate buffer.
MWO for 5 minutes at level 3
Cool for 1 minute (Add 50 mls citrate buffer to container)
MWO for 5 minutes at level 3. Temp after microwaving_____
Cool 20 minutes at room temperature
Rinse in distilled water 3 X 2 minutes each
Place slides in buffer for 5 minutes

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Block in 5% Normal Goat Serum for 20 minutes.
Lot#_____ Reconstituted Date_____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Apply Avidin/Biotin block
Lot#_____ New Kit yes / no
Apply avidin block - 15 min @ RT.
Quick rinse in 1X Automation Buffer.
Apply biotin block - 15 min @ RT.
No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Cox2) at 1:1000 dilution and incubate for one hour.
Lot#_____ Aliquoted yes / no Date Aliquoted_____

Comment: The concentration of this antibody varies significantly from lot-to-lot. It is advisable to test the dilution (1:10, 1:100, 1:1000) of a new lot on a positive control before doing a large run.

For the negative control slides, match the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (Cox2) and use this to make the 1:1000 dilution. Apply to the slides and incubate for one hour.
Lot #_____ Reconstituted Date_____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply secondary antibody (Biotinylated goat anti-rabbit) at 1:500 dilution and incubate for 30 minutes.
Lot#_____ Reconstituted Date_____

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Label antibody and incubate for 30 minutes. (Prepare 30 minutes prior to use)
Exp. Date _____ New Kit yes / no

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot #_____ Exp. Date_____ New Kit yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

17. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip
updated 01/14/2004